# Translocation of the threatened Growling Grass Frog *Litoria raniformis*: a case study.

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**ABSTRACT**

Translocation is occasionally suggested as a last resort strategy for dealing with 'unavoidable' loss of Growling Grass Frog *Litoria raniformis* habitat in urbanising landscapes. However, examples of attempts to translocate an entire population of *L. raniformis* are rare and their success (or lack of success) is poorly documented in the literature. In this study, we detail the translocation of a population of *L. raniformis* from a farm dam being destroyed for residential development to a purpose-built wetland 480 m away.

The population was translocated between November 2010 and May 2011. We used mark-recapture to estimate the number of frogs in the population prior to translocation. Visual counts of *L. raniformis* at the dam indicated a maximum of 39 adult frogs to be present while 355 frogs were marked over the course of a single season (November 2010 to March 2011).

Translocation of 156 frogs and unassisted colonisation by 32 frogs resulted in an estimated 70% of adults marked at the dam moving to the wetland and 91% of those remained there for the duration of the translocation study period.

The population and two measures of habitat quality (aquatic vegetation cover and water quality) were monitored for three active seasons post-translocation. Successful breeding was demonstrated for the first year only. A decline in breeding success was attributed to a reduction in habitat quality at the wetland, particularly the loss of submergent and floating vegetation due to the presence of Common Yabby *Cherax destructor*, a species that did not occur originally in the dam. We believe that colonisation of the wetland by this crustacean was due to the wetland being constructed on-line. An attempt to control the *C. destructor* population and re-establish the aquatic vegetation was unsuccessful.

We encourage the publication of all successes and failures in future attempts to establish translocated bell frog populations. If further experimental translocations have low success rates, then translocations should be reconsidered as a conservation strategy for *L. raniformis* in urbanising landscapes and greater emphasis placed on *in situ* habitat protection.

**Key words:** translocation, salvage, Growling Grass Frog, *Litoria raniformis*, mark-recapture, bell frog

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## Introduction

The Growling Grass Frog *Litoria raniformis* (Fig. 1) was once widespread and apparently very common in south-eastern Australia, with a distribution that included much of Victoria, southern New South Wales, the Australian Capital Territory, eastern Tasmania and south-eastern South Australia. However, the species has undergone a range contraction over the past 40 years and many populations are now either extinct or highly fragmented (Clemann and Gillespie 2012). The species is listed as Vulnerable under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and is listed as a threatened taxon in all States and Territories in which it occurs.

**Figure 1:** Growling Grass Frog *Litoria raniformis*. Photo: Daniel Gilmore.

The causes of the species' decline are thought to include habitat loss and modification, barriers to movement, disease (particularly chytridiomycosis caused by the introduced fungal pathogen *Batrachochytrium dendrobatidis*) and predation, especially by some introduced fish species (Clemann and Gillespie 2012; Heard *et al.* 2012a). Urbanisation in Melbourne's growth corridors is currently posing a serious threat to local *L. raniformis* populations, with many sites being lost in the past 20 years (Heard and Scroggie 2009).

Translocation is occasionally suggested as a last resort strategy for dealing with 'unavoidable' loss of *Litoria*

*raniformis* habitat in urbanising landscapes and there have been at least two instances where translocation has been undertaken in an effort to 'save' populations occupying wetlands earmarked for urban development. Although there are several unpublished reports on the subject (e.g. Clemann 2007; Robertson 2003; Wilson 2003), the process and outcomes of these translocations are poorly documented. It is therefore important to document attempts to translocate populations of *L. raniformis*, in accordance with the National Recovery Plan for the species (Clemann and Gillespie 2012).

Here we describe the translocation of a population of *L. raniformis* from a farm dam earmarked for destruction to a wetland constructed specifically to accommodate the population.

The objectives of the study were to:

i. translocate *L. raniformis* individuals to establish a population at the new wetland

ii. maximise the chances of successful establishment by translocating as many frogs and tadpoles from the source population as could be captured

iii. undertake the translocation as an experiment with a monitoring regime that would permit scrutiny and dissemination of the processes to inform future management decisions on behalf of the species.

# Methods

## Study site

The study took place in Victoria, Australia (Fig. 2). Approval to remove a farm dam supporting a population of *L. raniformis* to make way for residential development was granted under the Commonwealth EPBC Act. This was provided that, in an attempt to conserve the population, eight replacement wetlands were created online along Edgars Creek, an ephemeral stream running through the proposed development retained within an approximately 50 m wide corridor of open space (Fig. 3). To provide habitat for the translocated population, the first of these replacement wetlands was required to be constructed two active seasons prior to the removal of the dam. Construction of the first wetland was completed in 2009 and the dam was destroyed in 2011.

## Source site (dam)

The source site was a dam situated in farmland approximately 400 m west of Edgars Creek (144° 59' 5" E, -37° 36' 52" S) where a population of *L. raniformis* was discovered in 2002 (Costello *et al.* 2006). Compared to most other sites occupied by the species in the local area, this dam was relatively isolated from neighbouring populations, with the nearest known occupied site being

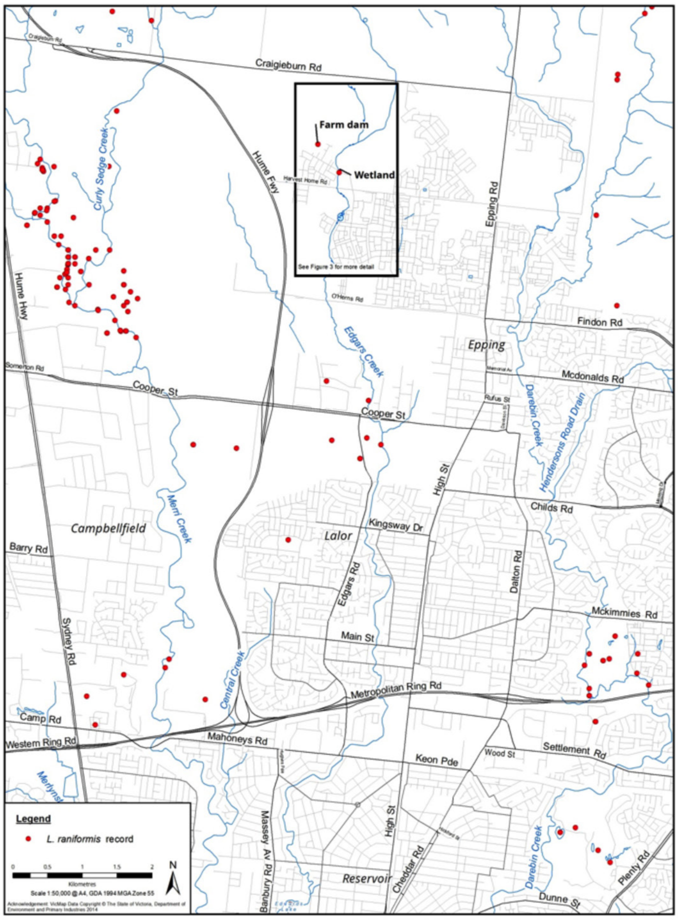


Figure 2: Landscape context of the study site.


**Figure 3:** Growling Grass Frog study site showing context of the source site (dam) and recipient site (wetland) and 'frog corridor.'

a constructed wetland 3.6 km to the south (Fig. 2). Nevertheless, the dam was a man-made waterbody, so it appears to have been naturally colonised by some dispersal event in the past and air photography shows the dam has existed since at least 1979.

The dam was thought to have been largely spring-fed through groundwater seepage (Costello *et al.* 2006; Thompson and Blackham 2011) but water levels may have been supplemented by a windmill and bore which was located in close proximity to the dam. Water levels within the dam were not monitored intensively between the time of the population's discovery in 2002 and its destruction in 2011, but it was noted to vary considerably both within and between years (Fig. 4). Examination of air photography from that period shows the water surface area varied from a minimum of approximately 170 m$^2$ to a maximum of approximately 500 m$^2$ during that time. Fluctuations in the water levels were likely due to variation in local rainfall, natural evaporative draw-down during summer and re-charge during winter and spring. On no occasion was the dam observed to dry out completely.

Vegetation cover within the dam varied in response to fluctuating water levels and seasonal growth of the dominant emergent macrophyte Salt Club Sedge *Bolboschoenus caldwellii* and the submergent/floating macrophyte Fennel Pondweed *Potamogeton pectinatus*, which senesced over winter and grew in the warmer months (Fig. 4).


**Figure 4:** Examples of the variable habitat condition of the dam: (a) September 2001 (winter condition; first time accessed), (b) September 2007 (winter condition plus cattle access after fence breach), (c) December 2009, note reduced area of water and growth of the emergent *Bolboschoenus caldwellii* (d) March 2010, (e) November 2010 following heavy rainfall that year that coincided with end of a prolonged drought event.

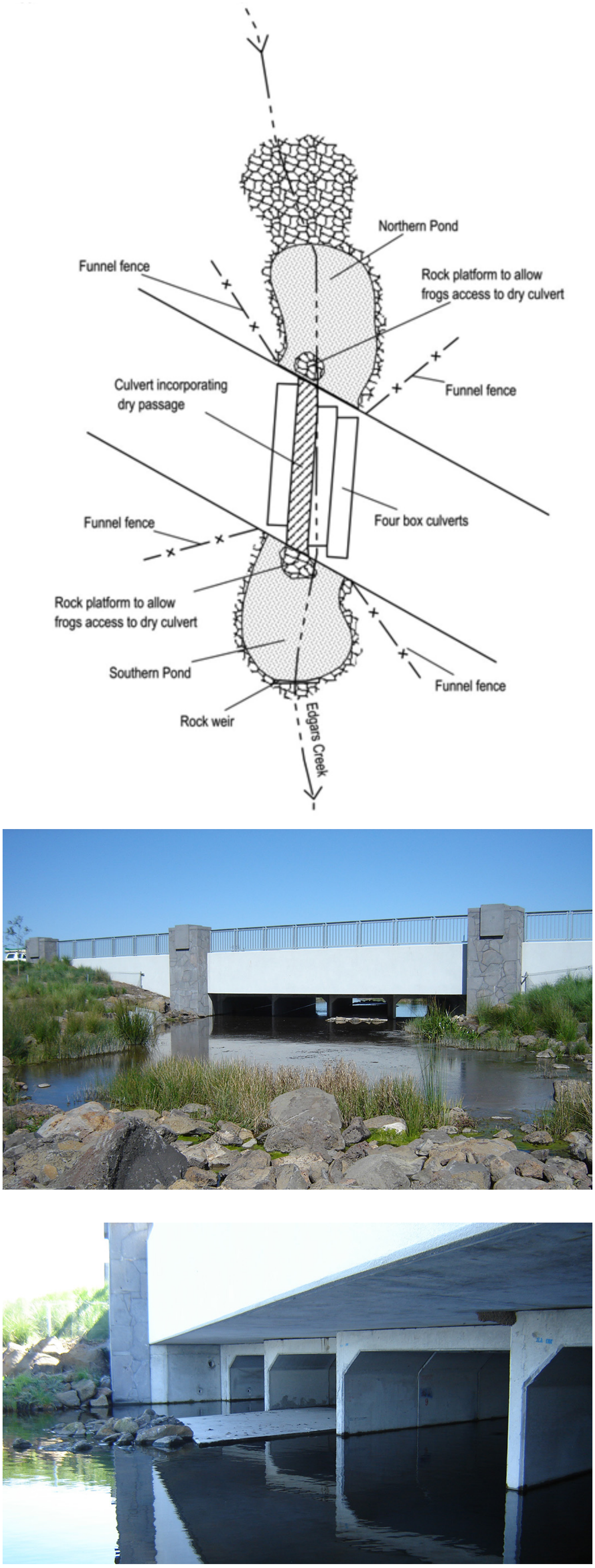


Figure 5: Created wetland: (a) design (adapted from plan produced by Spiire Pty Ltd) and (b) views of completed wetland. Photos: Sally Koehler.

## Recipient site (created wetland)

The wetland was created 480 m south-east of the dam (145° 0' 11" E, -37° 37' 6") at the point where a two lane municipal road crossed over the creek (Fig. 3). The wetland was constructed on a creek because it was considered likely to provide a reliable water source as urbanisation increases in the catchment – a permanent hydroperiod has been positively linked to *L. raniformis* wetland occupancy (Heard *et al.* 2010). The design incorporated structures to facilitate the movement of *L. raniformis* under the road (Koehler and Gilmore 2014) and habitat features favoured by *L. raniformis*, including a high cover and diversity of emergent, submergent and floating aquatic vegetation (Pyke 2002; Heard *et al.* 2008).

The wetland consists of two in-stream 'ponds' connected under the road by four 2.4 m W x 1.2 m H x 20 m L concrete box culverts (Fig. 5). Each pond has a water surface area of approximately 240 m$^2$ and a maximum depth of approximately 1.5 m. Funnel fences (17 m L x 0.8 m H) were installed on each side of both ponds to restrict frogs from moving onto the road and to funnel frogs towards the culvert entrances.

A 'frog-proof' fence was installed either side of a 100 m wide corridor to 'direct' dispersal of frogs from the dam to the wetland (Fig. 3). A 25 m wide strip within the corridor was mown when the biomass became dense as previous research has shown structurally open habitats are preferentially used by *L. raniformis* (Heard *et al.* 2008).

Construction of the wetland commenced in June 2009 and was completed (including wetland plantings) in September 2009. By November 2010, submergent and floating vegetation was well established in the wetland (Fig. 6).

## Pre-translocation monitoring

### *Habitat suitability*

We undertook an assessment of habitat suitability of the wetland in September 2010, 12 months after the new habitat was completed. The assessment was required to ascertain whether the wetland was suitable for occupancy by *L. raniformis* before translocation of the population could proceed. We subjectively deemed the wetland to be suitable for occupancy if emergent, submergent and floating aquatic vegetation had established and if water quality values in the wetland were similar to those at the dam.

In order to complete the assessment, we utilised data collected in relation to key habitat requirements of *L. raniformis* at the dam since 2002. This included information on percentage cover of emergent, floating and submergent vegetation within the waterbody and percentage cover of grass and shrubs (fringing vegetation), bare rock and bare ground within 5 m of the waterline.

Water quality was measured at the dam initially in March 2008, monthly from July 2008 to June 2009 (the first 12 months) and at six monthly intervals in spring and autumn until September 2010. Water quality monitoring in the wetland was undertaken in September 2009, March 2010 and September 2010 prior to translocation. *In situ* measurements were taken for Dissolved Oxygen (DO), pH,


Figure 6: Examples of the variable habitat condition of the created wetland: (a) 17 November 2010 – south of crossing, (b) 17 November 2010 – north of crossing, note dense cover of submergent and floating vegetation, (c) 8 February 2012 and (d) 20 November 2013. Note the loss of submergent aquatic vegetation and increased turbidity in 2013 compared to 2010.

Electrical Conductivity (EC), temperature and turbidity using a calibrated Horiba 52 water quality meter. All water quality measurements were undertaken in accordance with EPA Victoria guidelines for sampling and analysis of waters, wastewaters, soils and wastes (EPA 2009).

Water samples were also analysed for nutrient levels each spring and autumn, from spring 2008 to spring 2010 at the dam and in autumn 2010 at the created wetland. Analysis of Oxides of Nitrogen (NOx), Total Kjeldahl Nitrogen(TKN) and Total Phosphorus(TP) were conducted by a National Association of Testing Authorities accredited laboratory. *In situ* measures of alkalinity (CaCO3 (mg/L)) were also recorded using a HACH™ Alkalinity Testing Kit.

Predatory fish monitoring was not undertaken at the dam as it was not required under the EPBC Act approval for the development. However, no fish were recorded in the dam during our trapping for tadpoles using standard fish trapping methods.

### *Population monitoring*

We conducted *L. raniformis* counts at the dam when the population was first discovered in 2002 and then annually from 2007 to 2010. Count data were also collected independently by other ecologists annually between 2003 and 2008 (G. Heard and Ecology Australia unpubl. data) and their results have been incorporated into the results of this study.

To survey the population, we used the visual encounter method outlined by Crump and Scott (1994). Surveys involved a minimum of two people carefully searching the water surface and up to 5 m from the edge of the waterbody for nocturnally active frogs with the aid of head torches or spotlights. Surveys commenced after dark and lasted for 1 hour during which time *L. raniformis* were detected either by direct encounter or from their eye-shine. All surveys were timed to coincide with conditions that were optimal for detection of *L. raniformis* (above 12°C, with little or no wind). All surveys took place between September and April (inclusive), the species' primary active season in southern Victoria.

## Translocation program

### *Mark-recapture*

A salvage and translocation plan for *L. raniformis* was prepared in 2010 to inform the process (Koehler and Garvey 2010). This document was reviewed and accepted by the then Department of Sustainability and Environment, Victoria (now the Department of Environment and Primary Industries).

Because visual counts are likely to underestimate population size for bell frogs (Goldingay and Newell 2005), we considered a more robust population estimate would be beneficial prior to salvage. We used mark-recapture to more accurately estimate the size of the population

and to provide a measure against which to evaluate the proportion of the population that was collected and moved during translocation. This also allowed unassisted movements of translocated frogs between the dam and created wetland to be documented and success of the translocation to be more closely monitored through the ability to follow the movements of individual frogs. Mark-recapture has been undertaken successfully for the congeneric *L. aurea* (Goldingay and Newell 2005) and for *L. raniformis* elsewhere in the same catchment as our study (Heard *et al.* 2012a, b) and elsewhere in Victoria (e.g. Hamer and Organ 2008).

Searches for *L. raniformis* were conducted over five sessions of three consecutive nights on 28–30 November 2010 at the dam (session 1, nights 1–3), on 17–19 December 2010 (session 2, nights 4–6), 7–9 January 2011 (session 3, nights 7–9), 28–30 January 2011 (session 4, nights 10–12) and 15–17 March 2011 (session 5, nights 13–15) at both the dam and wetland using the methods outlined for population monitoring (above).

All adult *L. raniformis* were captured by hand and placed individually into plastic freezer bags with a small amount of water from the site. Each frog was weighed, measured (SVL) and sexed. Males were distinguished from females by the presence of nuptial pads and/or discoloured throats (Pyke 2002). All individuals >50 mm SVL were implanted with Trovan Ltd Passive Integrated Transponder (PIT) microchips using a technique similar to Christy (1996).

All frogs were handled in accordance with standard frog hygiene procedures available at the time (DEEC 2008).

### *Population modelling*

Closed population models (Otis *et al*. 1978) were used for analysis of the mark-recapture data to estimate abundance in each session (set of 3 consecutive nights). All frogs captured in the dam on the last nights of sessions 2, 3, 4 and 5 (nights 6, 9, 12, and 15) were translocated to the wetland (see below).

Program CAPTURE (White *et al.* 1978) was run from within Program MARK (http://www.phidot.org/software/mark/) to assess the assumptions of the closed population model for each session before fitting the appropriate models in MARK. A set of alternative models was fitted to the data from each session at each site and compared using the Akaike Information Criterion adjusted for small samples (AIC; Burnham and Anderson 2002) to select the best-fitting, parsimonious model.

### *Translocation of* L. raniformis

To minimise the number of individuals killed and injured during the filling in of the dam, and to try and maximise successful establishment at the recipient site, we attempted to translocate the entire population, including tadpoles. Translocation of *L. raniformis* from the dam to the wetland was undertaken in a staged manner rather than as a single translocation event and it commenced only once we considered an adequate cover of aquatic vegetation had established at the wetland.

Tadpoles were translocated on 17 November, 23 November, 3 December and 23 December 2010. Dip nets were used to capture tadpoles for the first three translocation sessions, although this was found to be problematic as the tadpoles tended to congregate in the deeper, open water section of the dam to evade capture. Several tadpoles caught in the dip nets were also found to have sustained tail injuries from the capture attempts. For these reasons seine and fyke nets were used for the final tadpole translocation and were found to be more efficient in this environment with minimal risk of injury or death to adults, metamorphs or tadpoles.

The seine net deployed was made of 6 mm cloth mesh and was 5 m wide and 1.25 m deep. A total of three hauls, each approximately 8–10 m in length were conducted, predominantly through the deep, open section in the middle of the dam. Three fyke nets, also consisting of 6 mm cloth mesh, were set over one night with a single, central wing with a drop of 1 m. Fyke nets were placed around the margin of the open section of the dam in 40–75 cm deep water. To prevent drowning of air breathing animals, nets were set to ensure an air pocket and floating vegetation was present at the top of each chamber. Captured tadpoles and source water were transferred to the wetland in plastic containers and released into submergent vegetation.

Translocation of adult and metamorph *L. raniformis* occurred on the third night of the four mark-recapture sessions. Mark-recapture sessions were timed to occur with ideal conditions for detection and capture *L. raniformis*, i.e. when the local weather forecast predicted three consecutive nights of minimum night-time temperatures above 12°C and with little or no wind. Minimum overnight temperatures were actually lower on some nights of translocations. Adult frogs were transferred to the created wetland separately in plastic bags. However, due to the large number of metamorphs encountered, it proved impractical to place all individuals of this age class separately into bags. It was therefore decided to place groups of metamorphs together and transfer them to the wetland. To avoid cannibalism (c.f. Pyke and White 2001), adults and metamorphs were never placed in the same bag.

A further two nights and two days of salvage and translocation occurred in the first week of May 2011 to capture any remaining individuals immediately prior to the destruction of the dam (small numbers of *L. raniformis* were still active and detectable at the dam during this time). Diurnal salvage was conducted by two people searching with the assistance of an excavator to lift rocks under which inactive frogs might be sheltering. Three fyke nets (described above) were set overnight on 3 May 2011.

### *Chytrid sampling and analysis*

To determine whether the fungal pathogen *B. dendrobatidis* that causes the disease chytridiomycosis was present in the population, skin swabs were taken during January 2011 from 33 frogs at the dam and 16 frogs at the wetland. Samples were collected using a sterile swab, which was rubbed over the ventral surface, inner thighs, and palms of the hands of each frog. Each sample was labelled and frozen before being sent to the CSIRO Australian Animal Health

Laboratory (AAHL) in Geelong for analysis using the PCR technique outlined by Hyatt *et al.* (2007). Additional swabs were taken from one dead and two sick frogs found at the wetland in May 2011 and were also sent for analysis.

## Post-translocation monitoring

### *Population*

Post-translocation surveys were conducted over three seasons (2011–12, 2012–13 and 2013–14). Visual counts were conducted on five occasions between October and March each year. During 2011–12, frogs were captured after each visual count. All frogs were handled in accordance with revised frog hygiene procedures outlined in Murray *et al*. (2011).

Frogs captured on the first survey night in October 2011 were implanted with PIT tags with the intention of continuing a less intensive (one night versus three) mark-recapture tagging program. However, on the basis of the low number of recaptures of frogs marked in the previous season we decided to abandon this additional PIT tagging.

During 2012–13 and 2013–14, hand capture was undertaken only on the first and last surveys because visual counts were considered to be sufficient to monitor population trends and the probability of capturing marked individuals was low.

Survey for tadpoles and predatory fish in the created wetland was undertaken three times per year between December and January since the wetland was established. Four fyke nets and ten funnel traps were deployed to detect tadpoles and predatory fish. All traps were set with a light stick (Cyalume Technologies) as an attractant and with adequate air spaces and floating vegetation to prevent drowning. Tadpole survey in January 2013 was supplemented with seine netting and dip netting to enhance tadpole detection.

### *Habitat suitability*

Water quality and the presence of predatory fish were monitored at the created wetland every 6 months following the same methods utilised during the pre-translocation monitoring stage.

A habitat assessment of the wetland was completed at the beginning and end of each season since 2009. The methods are the same as those described for pre-translocation monitoring.

### *Defining success of the translocation*

We deemed criteria for success of the translocation to be its capacity to move the majority of the population and whether the majority of frogs that were moved subsequently remained at the release site.

# Results

## Pre-translocation monitoring

### *Habitat suitability*

By September 2010, emergent, submergent and floating vegetation had established within the wetland (Table 1, Fig. 6) and water quality values at the two sites were broadly similar (Table 2). Hence, we considered that translocation could proceed.

**Table 1**. Vegetation assessment data for the dam and created wetland. Blank cells indicate habitat did not exist at the time of assessment.

| Date | Fringing vegetation (%) | | Aquatic vegetation | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Emergent (%) | | Floating (%) | | Submergent (%) | |
| | Dam | Wetland | Dam | Wetland | Dam | Wetland | Dam | Wetland |
| 11/09/2002 | 60 | | 20 | | 0 | | 90 | NP |
| Nov 2002* | 60 | | 25 | | 0 | | 90 | NP |
| Nov 2006* | 75 | | 25 | | 0 | | 90 | NP |
| 1/10/2009 | 75 | 0 | 40 | 0.5 | 7 | 0 | 3 | 0 |
| 22/12/2009 | 60 | 10 | 60 | 5 | 20 | 1 | 70 | 5 |
| 10/02/2010 | 25 | 50 | 90 | 15 | 5 | 10 | 5 | 5 |
| 14/09/2010 | 80 | 30 | 30 | 20 | 10 | 5 | 20 | 10 |
| 30/11/2010 | 75 | 35 | 55 | 20 | 5 | 50 | 10 | 0 |
| 15/03/2011 | 95 | 45 | 40 | 15 | 5 | 10 | 50 | 50 |
| 8/12/2011 | | 85 | | 10 | | 0 | | 50 |
| 29/03/2012 | | 70 | | 15 | | 7 | | 70 |
| 30/10/2012 | | 85 | | 30 | | 0 | | 0 |
| 6/12/2012 | | 85 | | 50 | | 20 | | 0 |
| 26/03/2013 | | 85 | | 20 | | 0 | | 0 |
| 20/10/2013 | | 85 | | 35 | | 0 | | 0 |
| 30/12/2013 | | 85 | | 55 | | 0 | | 0 |
| 20/03/2014 | | 80 | | 20 | | 0 | | 0 |

Notes to table: * data provided by G. Heard (Melbourne University).

**Table 2.** Water quality data for the dam and created wetland in spring 2008 to 2013*.

| Water quality parameter | Spring 2008 | Spring 2009 | | Spring 2010 | | Spring 2011 | Spring 2012 | Spring 2013 | Autumn 2014 |
|---|---|---|---|---|---|---|---|---|---|
| | Dam | Dam | Wetland | Dam | Wetland | Wetland | Wetland | Wetland | Wetland |
| pH | 8.32 | 7.88 | 7.15 | 8.20 | 7.75 | 8.76 | 8.45 | 7.58 | 7.97 |
| Turbidity (NTU) | 53.0 | 55.0 | 54.0 | 48.0 | 59.0 | 16.1 | 22.0 | 75.7 | 79.1 |
| EC (mS/cm) | 4.11 | 4.25 | 2.88 | 4.04 | 2.95 | 4.10 | 4.07 | 2.53 | 1.23 |
| DO (mg/L) | 8.74 | 7.86 | 5.95 | 5.57 | 8.84 | 17.35 | 10.88 | 7.31 | 5.33 |
| Temperature (°C) | 13.20 | 15.80 | 14.21 | 15.52 | 13.58 | 19.14 | 13.90 | 18.90 | 17.70 |
| TKN (mg N/L) | 1.6 | 2.4 | 2.0 | 1.6 | 1.4 | 1.7 | 2.1 | 1.3 | 1.0 |
| TP (mg P/L) | 0.058 | 0.140 | 0.077 | 0.088 | 0.190 | 0.130 | 0.086 | 0.085 | 0.087 |
| NOx (mg N/L) | 0.009 | <0.003 | 0.330 | 0.003 | 0.044 | 0.640 | 0.610 | 2.500 | 1.000 |

*Note: Results of autumn 2014 are also shown as they are the latest samples taken at the wetland.

### *Population monitoring*

The maximum visual count of *L. raniformis* prior to the commencement of the mark-recapture study was 39 at the dam in November 2010 (Fig. 7). Forty visual counts since 2002 had previously recorded maximum numbers of between 18 and 35 individuals on any given night of survey (Fig. 7). Evidence of breeding (presence of tadpoles or metamorphs) was confirmed to have occurred in the dam in every year between 2007 and 2010, when monitoring routinely encompassed the months from October to March (Fig. 8).

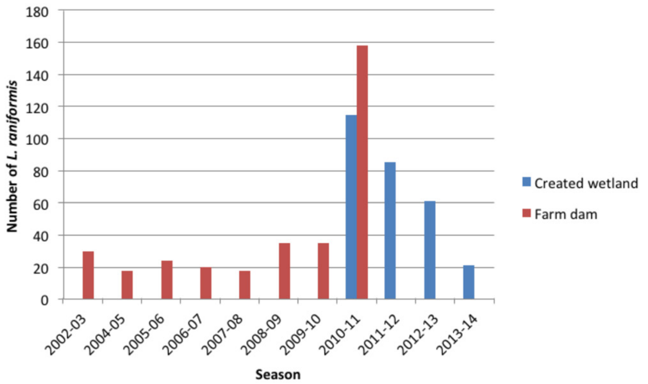


**Figure 7:** Maximum number of L. raniformis detected by visual encounter. The spike in numbers in 2010–11 is the result of an abundance of recently metamorphosed frogs which were present on the night of 7 January 2011.

A single male was heard calling in the created wetland in February 2010, 5 months after it was constructed and again in September and November 2010, prior to the commencement of the translocation. It is not possible to know whether these records were the same individual.

## Translocation program

### *Translocation of* L. raniformis

During the course of the study, a total of 355 frogs were marked. Of these, 269 individuals were marked at the dam and 86 were marked at the wetland (the latter group having colonised the wetland unassisted). The translocation history of marked frogs is presented in Table 3 and summarised below.

Of the 269 individuals marked at the dam, 156 (58%) were translocated to the wetland, while 32 (12%) colonised the wetland unassisted. Together this represents 70% of the population marked at the dam. Of the remaining 81 individuals marked at the dam whose fate could not be accounted for, 51 (63%) were never recaptured and 30 (37%) were recaptured at least once but not on a translocation night (and hence were not translocated to the wetland). Thus, by the end of the translocation there

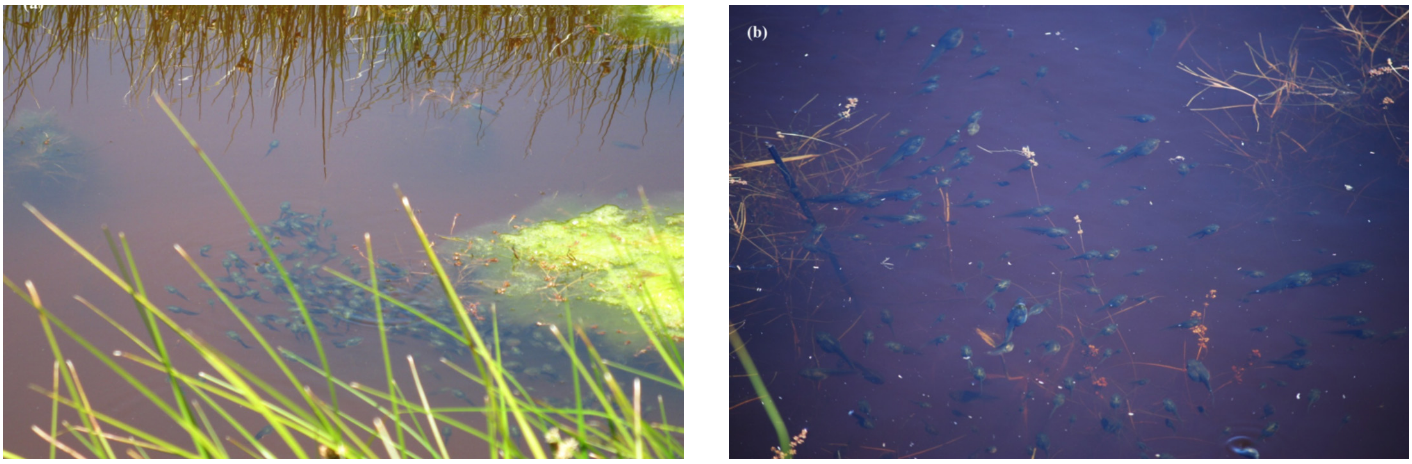


**Figure 8.** *L. raniformis* tadpoles at the dam in December 2009 and November 2010. Note the schooling behaviour, which has also been reported in the closely related *L. aurea*.

**Table 3:** Translocation outcomes of marked frogs

| First marked at | Translocation outcome | Total |
|---|---|---|
| Dam | Translocated (did not return to dam) | 142 |
| | Translocated (returned to dam, not translocated again) | 2 |
| | Translocated (returned to dam, translocated a 2nd time) | 10 |
| | Translocated (returned to dam, translocated a 2nd and 3rd time) | 1 |
| | Translocated (returned to dam, returned to created habitat unassisted) | 1 |
| | Not translocated (not recaptured after first being marked) | 51 |
| | Not translocated (not captured on a translocation night) | 30 |
| | Not translocated (marked at dam, found created habitat unassisted) | 32 |
| Wetland | Not translocated (natural colonisation) | 86 |
| **Total marked** | | **355** |

were 274 marked individuals at the wetland.

In addition to marked frogs, we also translocated 324 individuals <50 mm SVL and approximately 1,900 tadpoles over four days and five nights between November 2010 and May 2011.

*Post-translocation movements*

Fourteen individuals (9% of the marked frogs translocated to the wetland) were recorded moving back to the dam. We did not record any of the 118 frogs that colonised the wetland unassisted moving to the dam.

*Chytrid testing*

All *L. raniformis* swabbed in January 2011 tested negative for chytrid fungus. Swabs of one dead and two sickindividuals collected at the wetland in May 2011 tested positive.

*Population estimates*

Prior to translocation, the size of the population of *L. raniformis* in the dam was estimated at 235 frogs (95% confidence interval, 170–348 individuals) when 100 frogs were captured during the first session. This estimate was refined by the second session to 208 frogs (95% confidence interval, 183–248 individuals) based on a cumulative total of 189 frogs marked at the dam prior to translocation commencing (Table 4). Conversely, the population estimated at the created wetland prior to translocation commencing was eight

**Table 4:** Numbers of frogs newly marked in each session and translocated on the third night of each session, closed population models used, the estimated capture probabilities (P hat), and the estimated abundance (N hat) with its 95% confidence interval (L95N hat, U95N hat) for each session at each site.

| Site | Session | Number newly marked | Number translocated on night 3 | Model[1] | Time (night) | P hat | N hat | L95N hat | U95N hat |
|---|---|---|---|---|---|---|---|---|---|
| Dam | 1 | 100 | 0 | M(t) | 1 | 0.07 | | | |
| | | | | | 2 | 0.15 | | | |
| | | | | | 3 | 0.28 | 235 | 170 | 348 |
| | 2 | 89 | 58 | M(0) | all | 0.32 | 208 | 183 | 248 |
| | 3 | 31 | 54 | M(0) | all | 0.38 | 126 | 112 | 149 |
| | 4 | 15 | 21 | M(0) | all | 0.19 | 84 | 55 | 160 |
| | 5 | 34 | 23 | M(0) | all | 0.30 | 73 | 60 | 105 |
| Wetland | 2 | 5 | 58 | M(0) | all | 0.33 | 8 | 6 | 32 |
| | 3 | 21 | 54 | M(t) | 1 | 0.52 | | | |
| | | | | | 2 | 0.15 | | | |
| | | | | | 3 | 0.32 | 106 | 90 | 135 |
| | 4 | 13 | 21 | M(th) | 1 | 0.14 | | | |
| | | | | | 2 | 0.25 | | | |
| | | | | | 3 | 0.21 | 181 | 118 | 362 |
| | 5 | 47 | 23 | M(th) | 1 | 0.21 | | | |
| | | | | | 2 | 0.10 | | | |
| | | | | | 3 | 0.16 | 232 | 146 | 461 |

[1]Model notation is that employed by Otis *et al.* (1978). M(0) indicates that capture probabilities were constant over the three nights in the session, M(t) indicates that capture probabilities varied by night, M(th) indicates that capture probabilities varied both by night and individual frog.

(95% confidence interval, 6–32 individuals).

Of 235 frogs estimated to be present at the dam in session one, 133 were translocated prior to session five when 73 were estimated to still be present. Of these, 23 frogs were translocated on the third night of session five, leaving an estimated 50 frogs remaining at the dam when translocation ceased.

Eight frogs were estimated to be present at the wetland in session two prior to translocation, 133 frogs were added between the third night of session two and the third night of session four. The estimated abundance in session five was 232 frogs prior to the addition of 23 translocated frogs on the third night of session five, leading to an estimated 255 frogs present at the wetland (Table 4).

## Post-translocation monitoring

### *Population*

The maximum number of frogs observed at the wetland during a single night in 2011–12 was 85, and 69 were captured during the searches (Fig. 7). None of the 355 frogs marked during the 2010–11 season were recaptured in 2011–12. A maximum of 86 tadpoles and two metamorphs were captured in the wetland during survey, suggesting that breeding had occurred.

The maximum number of frogs observed during a single night in 2012–13 was 61, and 35 were captured during the searches. Individuals less than 50 mm SVL were captured during survey in October 2012. No tadpoles were recorded.

Two marked frogs were recaptured during the 2012–13 season. One of the 18 frogs marked at the created wetland early in the 2011–12 season (a male) was recaptured in October 2012. A female marked at the dam on 29 November 2010 was captured again at the wetland on 30 October 2012 and 26 March 2013. Based on the date she was first captured and her size at the time (60 mm SVL and 18 g), she was likely recruited into the population during or before the 2009–10 season. Thus, she was at least three years of age when last recaptured in March 2013 and had grown to 74 mm SVL and weighed 76 g. She was the only marked frog recaptured from the 2010-11 season translocation and mark-recapture study.

In 2013–14, a maximum of 21 frogs was captured during survey, 65% fewer than the previous season. No marked frogs were captured and no tadpoles or metamorphs were recorded.

## Habitat suitability post-translocation

Vegetation monitoring of the wetland identified a loss of submergent and floating vegetation in the 2012–13 season while the cover of emergent vegetation increased and has remained stable (Table 1). Submergent and floating vegetation was still absent at the wetland despite an attempt to re-establish this habitat component.

Water quality monitoring indicated that all water quality values measured fluctuated but remained generally consistent over time at the wetland and were broadly comparable with the dam prior to its destruction (Table 2). There were a few exceptions. Electrical conductivity, while remaining reasonably constant, was generally lower at the wetland compared to the dam. The lowest level of electrical conductivity to date (1.23 mS/cm) was recorded in autumn 2014. Levels of nitrates / nitrites (oxides of nitrogen) were generally higher at the wetland than those recorded in the dam and increased over time. The 400% increase in levels of nitrites / nitrates experienced in spring 2013 followed an extensive grassfire in February 2013. While the most recent results (autumn 2014) indicate levels have returned to a more usual level for the created habitat, they remain 60% higher than the levels pre-fire. Turbidity levels in the wetland continued to increase throughout the monitoring period with the highest value recorded in autumn 2014.

Inputs of urban waste (litter including garden waste), yabby traps, building materials (plastics and polystyrene) and pollutants associated with road run-off were consistently observed at the recipient site. Site maintenance crews have removed the bulk of the coarse pollutant material over time.

No exotic fish were recorded within the created wetland. A single native Short-finned Eel *Anguilla australis* was captured in March 2011, December 2011 and February 2012 and observed in January 2013.

Common Yabby *Cherax destructor* was first detected in the created wetland in low numbers (27) during tadpole and predatory fish sampling in 2011–12. Numbers of *C. destructor* increased with 185 individuals of varying age classes captured in January 2013. This increase coincided with an absence of breeding and recruitment by *L. raniformis* and a loss of submergent and floating vegetation. As *C. destructor* is known to consume mainly plant material (Linton *et al.* 2009) and to increase water turbidity (which in turn may affect the growth of aquatic plants), we considered it necessary to intervene and implement a control program. On this basis, we developed a habitat improvement plan in consultation with Department of Environment and Primary Industries incorporating removal of *C. destructor* and supplementary planting of submergent and floating macrophytes (Koehler *et al.* 2013). The plan was implemented in 2013 prior to the next active season of *L raniformis*. Removal of *C. destructor* was undertaken in August 2013 using a combination of four 1 m drop nets and a seine net. Drop nets were utilised while the water level in the wetland was lowered. Baited drop nets were deployed for up to 60 minutes. A seine net was used once water depth was subsequently lowered to 600 mm. Hauls were repeated until the numbers of *C. destructor* recorded in each haul was consistently less than 10 individuals.

A total of 3,495 *C. destructor* of various sizes (OCL 10–100 mm) was removed from the wetland in August 2013. All *C. destructor* collected were euthanised using Aqui-S anaesthetic.

Supplementary planting to restock the wetland with aquatic macrophytes occurred in September 2013 after removal of *C. destructor* was completed. These plants failed to establish.

## Discussion

Visual encounter surveys prior to marking underestimated the number of frogs in the population. The maximum number detected prior to marking commencing was 39, while 269 frogs were marked during our mark-recapture program at the dam. Large differences in population estimates derived from visual counts versus mark-recapture data have been previously documented for bell frog populations, with visual counts often severely underestimating population size.

Population modelling was useful to estimate the number of frogs in the population and therefore how many to translocate to achieve the aim of moving the majority of them. Modelling at the end of the fifth translocation session estimated there were 50 individuals remaining at the dam. The final salvage of the dam in May 2011 resulted in the capture an additional 42 individuals, which compares well with the modelling estimate.

There are few estimates of population sizes of bell frogs but the estimate for the Wollert population is within the range of population sizes published for other species of bell frog (Goldingay and Newell 2005; Mahony *et al.* 2013).

### Was assisted movement to the replacement habitat necessary?

The aim of the translocation was to establish a self-sustaining *L. raniformis* population in the created wetland. To achieve this, the population needed to colonise the new habitat either through natural dispersal, assisted movement, or both. This study demonstrated that 118 *L. raniformis* inhabiting the dam naturally dispersed to the created wetland 480 m away, albeit that movements were 'directed' through the installation of a 100 m wide fenced corridor. It is not known how much influence this fence had on directing frogs to the wetland. The time that our translocation program commenced coincided with the wettest season experienced in Melbourne following the end of a 14 year drought (BOM 2010) and this may have increased the capacity for *L. raniformis* to disperse to the created wetland. It is possible that once males began chorusing at the wetland in November and December 2010 they attracted other frogs from the dam.

It is not known to what extent translocation increases the likelihood that a population of *L. raniformis* will successfully establish at constructed wetlands over and above natural colonisation. Indeed, each situation is likely to vary depending on site context, population size and productivity, the time lag between the new habitat being created and the original habitat being destroyed and, importantly, the distance between the original and created habitat (Heard *et al.* 2010).

Due to the isolation of the farm dam and the fact that it was earmarked for destruction, it was considered best to attempt to relocate as many of the resident *L. raniformis* as possible. This was the primary reason we carried out translocation rather than relying solely on natural colonisation from the dam. We also thought that translocating tadpoles from the dam might increase the probability of successful establishment at the new site, as we hypothesised that tadpoles metamorphosing at the wetland might display site fidelity and remain to ultimately breed there. In addition, these tadpoles were viewed as potentially very important as they would form the bulk of the breeding population the following year.

### Did the population establish?

Our results show that 70% of all *L. raniformis* marked at the dam were either translocated to the wetland, or colonised it naturally and that 91% of those individuals remained there for the four months of intensive monitoring during the mark-recapture study.

The observation of tadpoles in 2011–12 leads us to believe that successful breeding did occur in the first season following translocation but the possibility cannot be discounted that they were actually translocated tadpoles that had overwintered from the previous season. The larval lifespan for *L. raniformis* is described as being between 2 and 15 months (Anstis 2013; Clemann and Gillespie 2012). However, it is believed the majority of *L. raniformis* tadpoles complete metamorphosis within a single breeding season, with tadpoles rarely, if ever overwintering (Cree 1984; Mann *et al.* 2010).

In the second season no evidence of breeding was found during targeted tadpole surveys. The presence of small metamorphosed individuals (<50 mm SVL) observed early in October 2012 suggests either low levels of metamorphosis from overwintering tadpoles or late metamorphosing individuals from the previous season that experienced limited growth over the winter.

Breeding was not observed during 2012–13 or 2013–14. The cause of this failure to breed is unclear, but factors known or suspected to influence breeding success in other translocated bell frog populations include poorly designed replacement habitat, habitat deterioration, poor water quality, predation and disease (Pyke and White 2001; Wilson 2003; Clemann 2007; White and Pyke 2008).

Predation by introduced fish such as Plague Minnow *Gambusia holbrooki* (Gillespie and Hero 1999; Pyke and White 2000; Pyke 2002; White and Pyke 2008) can be discounted as the cause of breeding failure as no introduced fish were present in the wetland. Other potential predators of eggs and tadpoles such as eels, snakes and waterbirds were observed occasionally at the wetland but the extent to which they predated upon *L. raniformis* is not known.

Sampling of *L. raniformis* at the wetland confirmed the presence of chytrid in the translocated population and chytridiomycosis is likely to be a source of mortality there, particularly during the cooler months (Berger *et al.* 2004). Chytridiomycosis was attributed to the decline of a reintroduced population of *L. aurea* in the Hunter Region of New South Wales (Stockwell *et al.* 2008) and mortalities from this disease may be partly responsible for the virtual lack of recaptures of marked frogs in our study.

In the Merri Creek corridor north of Melbourne, Heard *et al.* (2014) found water temperature and salinity in wetlands both had negative effects on the probability and

intensity of chytrid infection in *L. raniformis* and suggested that warm and saline wetlands may act as refugia from chytridiomycosis for *L. raniformis*. However, it is not known if chytrid prevalence or the intensity of infections differed between the wetland and the dam. Salinity levels in the wetland were generally lower than the dam so it is possible that chytrid infection rates may have been higher at the wetland as a consequence. Unlike the dam, which was spring-fed from relatively saline groundwater (Thompson and Blackham 2011), the wetland is on an ephemeral creek and receives freshwater surface runoff that may reduce salinity.

Unless chytridiomycosis reduced the health of sexually mature frogs at the wetland to the extent that they were unable mate and lay eggs, it is unlikely to be responsible for the absence of tadpoles because, although they are susceptible to chytrid infection, they do not appear to succumb to chytridiomycosis (DEH 2006).

Little is known about the effects of *C. destructor* on *L. raniformis* or its habitat. Decline of amphibian populations in streams and dams in New South Wales and mortality associated with predation of frog eggs and tadpoles has been attributed to *C. destructor* (Coughran *et al.* 2009; Coughran and Daly 2012). *Cherax destructor* is known to consume and/or destroy aquatic macrophytes and resuspend sediments through their burrowing and foraging behaviours (Dorn and Wojdak 2004; Linton *et al.* 2009). Like other members of the bell frog complex, *L. raniformis* relies heavily on the presence of floating and submergent plants as calling platforms for males, oviposition sites and as shelter and food for tadpoles (Pyke 2002; Hamer and Organ 2008; Heard *et al.* 2008). It is therefore likely that the loss of this critical habitat resource contributed to the breeding failure in the translocated *L. raniformis* population. Attempts to re-establish submergent and floating vegetation were unsuccessful.

*Cherax destructor* may also prey upon *L. raniformis* eggs and larvae and this may also be a contributing factor. Predation of eggs and stalking of live prey including frogs, tadpoles and fish are known feeding behaviours of various aquatic crustaceans (Gherardi *et al.* 2001; Riley *et al.* 2005; Coughran and Daly 2012; Ficetola *et al.* 2012; Pyke *et al.* 2013). The egg masses of *L. raniformis* sink to the bottom of the waterbody after oviposition (Anstis 2013), so egg masses may be vulnerable to predation by *C. destructor*.

Yabbies are difficult to control and are well known for their capacity to proliferate to high densities and attain maturity in as little as four months (Coughran *et al.* 2009). An attempt to temporarily reduce the number of *C. destructor* in the lead-up to the 2013–14 *L. raniformis* active season was unsuccessful as numbers rapidly returned to pre-control levels.

Water quality parameters within the created wetland remained within ranges known to be tolerated by *L. raniformis* (Pyke 2002; Heard *et al*. n.d.). However, an increase in turbidity and levels of nitrites / nitrates coinciding with the presence and proliferation of *C. destructor* and, to a lesser extent, an increasingly urbanised catchment and associated stormwater inputs, may be contributing to a decline in habitat suitability at this site. We consider that the decline in habitat quality is largely a consequence of the wetland being constructed on-line, adjacent to a road and in an increasingly urbanised area (Riley *et al*. 2005; Hamer and McDonnell 2008; Hamer and Parris 2011).

## Future prospects for the Wollert population

Monitoring of the translocated *L. raniformis* population at Wollert is intended to continue until July 2033 in line with the EPBC approval for the development. The approval requires the establishment of a total of eight wetlands along Edgars Creek. The first of those is the wetland site discussed here and one further wetland was constructed in 2013. The EPBC Act approval deadline for completion of the next three wetlands is 2016. It is hoped that once the additional wetlands proposed along the creek have been constructed there will be an overall increase in habitat which may reduce the likelihood of localised extinction (Hale *et al.* 2012; Heard *et al.* 2012b).

Habitat connectivity for *L. raniformis* along the creek will be maintained through demonstrably effective culvert design (Koehler and Gilmore 2014) and open space managed to facilitate movements by the species. Nonetheless, we recognise that poor habitat quality is a real cause of decline of amphibians in urban landscapes (Hamer and Parris 2011) and is a primary reason for failure of translocations of herpetofauna (Germano and Bishop 2009). We also recognise the reality that urbanisation in the area of our study may result in ultimate decline of *L. raniformis* there and our study provides details of specific difficulties faced by translocation efforts within an urbanising environment. Results of the study are now being used to evaluate revised options for conservation of the species at Wollert.

## How does this experience compare with other bell frogs translocations?

Apart from extralimital introductions to New Zealand (Bishop 2008), and possible introductions in the Mt Lofty Ranges and the Adelaide Plains of South Australia (Clemann and Gillespie 2012), only two translocations of *L. raniformis* have been undertaken and both were in response to imminent habitat destruction. The first involved the removal of a disused quarry in Melbourne's south-east that supported a large population. Prior to destruction of the site, 50 *L. raniformis* were salvaged and released into a well-vegetated stormwater wetland at Waterways, a residential estate approximately 10 km away (Robertson 2003; Clemann 2007). The translocated frogs were monitored sporadically after the release in January 2002, but no *L. raniformis* have been recorded at the release site since January 2006 and the population apparently failed to persist (Smith and Clemann 2008; D. Gilmore, pers. obs.).

At another site at Bundoora in Melbourne's north, 42 *L. raniformis* (adults and sub-adults) were translocated from a disused quarry hole earmarked for removal to a stormwater treatment wetland 500 m away. The quarry hole was within close proximity to Darebin Creek and the two other quarry holes inhabited by the species. The

new wetland was designed to provide alternative habitat for the species to partially offset the loss of the quarry hole (Wilson 2003). Monitoring demonstrated that *L. raniformis* colonised and successfully bred at the wetland prior to the translocation (Heard *et al.* 2004). Darebin Creek and the two remaining quarry holes, both of which still support *L. raniformis*, are within the dispersal range of the species and are likely to have been important for the establishment and persistence of the population as has been shown for translocated population of *L. aurea* (White and Pyke 2008). Although the extent of suitable breeding habitat at this site has declined due to excessive growth of Common Reed *Phragmites australis* and Cumbungi *Typha* sp. successful breeding has occurred at the wetland created for this project as recently as 2012–13 (D. Gilmore, pers. obs. 2013).

Since there is the capacity for ongoing recolonisation from key breeding habitat nearby, it is unclear whether the population in the created wetland in Bundoora is self-sustaining. Therefore it is not comparable to the situation at Wollert, which is relatively isolated from other *L. raniformis* populations and where the known breeding dam has now been destroyed, precluding the opportunity for ready population supplementation and recolonisation.

Superficially *L. raniformis* appears ideally suited to translocation to created habitats as, like the closely related Green and Golden Bell Frog *Litoria aurea,* the species has naturally colonised a range of artificial waterbodies with long-term success (Pyke and White 2001; Heard *et al.* 2008; Ramamurthy and Coulson 2008; White and Pyke 2008). However, despite numerous attempts to translocate bell fogs, they often fail.

The problems encountered during our study are similar to those experienced with translocations and reintroductions of *L. aurea* in New South Wales (Daly *et al.* 2008; Pyke *et al.* 2008; White and Pyke 2008). They highlight the risky nature of this management approach for bell frog populations.

### Implications for management of *L. raniformis* in urbanising environments

Populations of *L. raniformis* are scattered throughout the growth areas of Melbourne and the long-term conservation of populations of the species in the urban environment has received considerable attention (Heard *et al.* 2010). As part of Melbourne's urban development program, a strategy has been prepared by the State of Victoria as part of a Strategic Assessment under the EPBC Act (DEPI 2013). The main focus of this strategy is to permanently protect and manage important populations along major drainage lines within the growth areas (so-called Category 1 habitat) while permitting the removal of habitat deemed to be of lesser significance to the species (Category 2 habitat) (Ecology and Heritage Partners 2011; Gilmore and Shepherd 2012; DEPI 2013).

With this approach, the removal of Category 2 habitat can occur, provided compensatory habitat is provided elsewhere – generally through the construction of dedicated wetlands in Category 1 habitat. Salvage and translocation of 'doomed' populations of *L. raniformis* within Category 2 habitat to appropriate created habitat in Category 1 habitat is proposed as part of the strategy (DEPI 2013). It is therefore important to be well informed about the effectiveness of salvage and translocation for this species.

Clearly, the success of any attempt to translocate this species will be highly dependent on the quality of the habitat at the recipient site. While a wetland created in an urban area in close proximity to an existing *L. raniformis* population may rapidly establish important habitat attributes such as submergent and floating vegetation, and be readily colonised by the species, its suitability for the species in the longer term is likely to be strongly influenced by its position in the landscape. We consider the deteriorating habitat quality and various other negative effects on the species following an initial phase of apparent success observed in the wetland in our study can be attributed to its situation on a creek.

Our study documented the process and logistics of translocating a population of *L. raniformis*, including the considerable effort required, and highlights the inherent risks involved. We encourage the publication of all successes and failures in future attempts to establish translocated bell frog populations. If further experimental translocations have low success rates, then translocations should be reconsidered as a conservation strategy for *L. raniformis* in urbanising landscapes and greater emphasis placed on *in situ* habitat protection.

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